

Below-ground causes and consequences of woodland shrub invasions: a novel paired-point framework reveals new insights

Basil V. Iannone III^{1*}, Liam Heneghan², Dev Rijal³ and David H. Wise^{3,4}

¹Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907, USA; ²Department of Environmental Science and Studies, DePaul University, Chicago, IL 60614, USA; ³Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60607, USA; and ⁴Institute for Environmental Science and Policy, University of Illinois at Chicago, Chicago, IL 60612, USA

Summary

1. Confirming the impacts of invasive plants is essential for prioritizing management efforts, but is challenging, especially if impacts occur below-ground and over long periods as hypothesized for woodland shrub invasions.

2. For this reason, we developed a novel ‘paired-point’ framework capable of detecting the below-ground impacts of slow-growing invasive plants from short-term data sets in an investigation into the below-ground consequences of European buckthorn *Rhamnus cathartica* L. (hereafter buckthorn) invasions into Chicago-area woodlands. We measured differences between the members of 97 pairs of buckthorn-invaded and buckthorn-free points in 10 below-ground response variables (RVs) that buckthorn is hypothesized to alter (listed below). We then modelled these differences (Δ RVs) in response to the severity of the invasion found at each invaded point. A relationship (linear or nonlinear) between a Δ RV and severity, that is slope $\neq 0$, suggests a buckthorn-induced change. An intercept (value of Δ RV where severity = 0) different from zero suggests a pre-existing difference.

3. Relating differences to an invasion gradient rather than simply noting their presence provided evidence that the higher levels of moisture, pH, total C, total N, $\text{NH}_4^+ - \text{N}$ and Ca^{2+} in invaded soils pre-date and possibly promote invasion (particularly Ca^{2+}) and that neither earthworm biomass nor soil C : N ratios are associated with buckthorn invasion, all of which suggest buckthorn to be less of a ‘driver’ of below-ground change than hypothesized. We did, however, find evidence that buckthorn establishes in areas having greater leaf litter mass and higher rates of decomposition, and then proceeds to accelerate decomposition further and to alter spring soil $\text{NO}_3^- - \text{N}$ levels.

4. *Synthesis and applications.* Our findings suggest that decisions regarding regional buckthorn management should be less driven by concerns about buckthorn’s below-ground impacts and that greater consideration of how variation in below-ground factors relates to invader establishment is needed, rather than simply assuming this variation to be invader-induced. This latter consideration can help to design better targeted monitoring programmes, limiting the further spread of woodland invaders. These insights illustrate the utility of the paired-point framework both for investigating below-ground causes and consequences of slow-growing invasive plants and for guiding the management of these invasions.

Key-words: above-ground–below-ground interactions, causes of plant invasion, European buckthorn, exotic shrubs, invasion impacts, leaf litter inputs, natural experiments, *Rhamnus cathartica* L., soils, woodland management

*Correspondence author. E-mail: biannone@purdue.edu

Introduction

The potential for invasive plants to alter soils is well-established (Ehrenfeld 2003; Liao *et al.* 2008). These below-ground changes can affect native plant communities via multiple pathways (Goldberg 1990; Levine *et al.* 2003; Callaway & Ridenour 2004; Bever *et al.* 2010) and, in some cases, continue to do so beyond invasive species removal (i.e. during ecological restoration) (Corbin & D'Antonio 2004), contributing substantially to the invader's overall impact (*sensu* Parker *et al.* 1999; Ricciardi *et al.* 2013). Although invasive plants clearly can have strong ecological impacts, the detection and confirmation of invader-induced effects is challenging, particularly for those occurring below-ground and at the ecosystem level (Parker *et al.* 1999). The challenge comes from the frequent absence of data on pre-invaded conditions, and from the fact that the effects of invasive plants vary both spatially and temporally (Hulme *et al.* 2013). Overcoming the difficulties of convincingly establishing invasive-induced effects is, however, essential to the prioritization of management efforts (e.g. eradication efforts, pre-restoration soil remediation, etc.) (Parker *et al.* 1999; Kardol & Wardle 2010).

Exotic shrubs constitute a subset of invasive plants that may profoundly affect community- and ecosystem-level properties (Gaertner *et al.* 2014; Rejmánek 2014). It is hypothesized that such strong effects are due to the structural and biogeochemical properties of invasive shrubs relative to the species in the systems where they invade (Rejmánek 2014), that is to their unique functionality (Levine *et al.* 2003; Ricciardi *et al.* 2013). While shrub invasions occur globally, they are particularly prevalent, and problematic, in the temperate woodlands of North America (Martin, Canham & Marks 2009; Rejmánek 2014). Some of the shrub species invading this region are further hypothesized to alter soil properties via their high-quality leaf litter (Ehrenfeld, Kourtev & Huang 2001; Ashton *et al.* 2005; Heneghan *et al.* 2006; Heneghan, Steffen & Fagen 2007). Such changes are implicated as factors causing drastic regime shifts, potentially making the control of these invaders a top management priority (Gaertner *et al.* 2014); however, land managers have limited resources, which means that these below-ground changes must first be confirmed prior to making management decisions.

Detecting below-ground effects of slow-growing invasive shrubs is particularly difficult for two reasons, the first of which is the decadal time-scales over which their invasions occur (Martin, Canham & Marks 2009). Below-ground changes may accumulate too slowly to be detected by short-term manipulative experiments. While long-term common-garden studies can detect such changes (e.g. Reich *et al.* 2005), they have not been conducted for North American shrub invasions, and they may not generate information quickly enough to guide management and research. Even manipulative experiments under

controlled settings such as greenhouses present problems of interpretation. Although these studies reveal that exotic shrubs can alter soils in simplified environments (Ehrenfeld, Kourtev & Huang 2001; Kourtev, Ehrenfeld & Häggblom 2003), in the field these effects may not occur, or may be undetectable (Elgersma *et al.* 2011).

The second challenge is establishing the causal basis of differences in properties of invaded and uninvaded soil. Some authors have compared differences between woodland soils with and without exotic shrubs to infer the below-ground consequences of these invaders (Kourtev, Huang & Ehrenfeld 1999; Ehrenfeld, Kourtev & Huang 2001; Heneghan *et al.* 2006; Heneghan, Steffen & Fagen 2007). Although observed differences are consistent with shrub-induced change, an equally plausible explanation is that differences pre-dated, and possibly promoted, invasion. This latter interpretation is supported by the fact that many below-ground factors that exotic shrubs are hypothesized to affect may also promote the establishment of aggressive plants (Wardle 2002; Gilbert & Lechowicz 2005; Frelich *et al.* 2006; Gilliam 2006; Gurevitch *et al.* 2008).

These complications illustrate the need for empirical methods that utilize short-term data sets to derive inferences about possible long-term effects of invasive shrubs and that can also help to distinguish shrub-induced changes from pre-invasion differences. Such a method would improve our understanding of the overall impact (*sensu* Parker *et al.* 1999) of invasive shrubs and other slow-growing, woodland plant invaders (Martin, Canham & Marks 2009), thereby helping to prioritize management efforts (Parker *et al.* 1999; Kardol & Wardle 2010).

Here we report the findings generated by one such method – a 'paired-point' framework – that we developed to investigate the below-ground consequences of the slow-growing, invasive shrub European buckthorn *Rhamnus cathartica* L. (hereafter buckthorn) in remnant Chicago-area woodlands. Buckthorn has invaded a large portion of North America (Kurylo *et al.* 2007) where it has become a concern for woodland conservation and is hypothesized to alter many soil properties via its high-quality leaf litter (Heneghan *et al.* 2006; Heneghan, Steffen & Fagen 2007). This hypothesis, however, is based on a relatively few observed differences between woodland soils with and without buckthorn. These differences may reflect pre-invaded conditions that promote invasion rather than buckthorn-induced changes. Nonetheless, 16 articles citing these two studies (i.e. Heneghan *et al.* 2006; Heneghan, Steffen & Fagen 2007) state as fact that buckthorn causes these differences (Google Scholar search), and two meta-analyses into the overarching effects of invasive plants include these studies in their data base (Liao *et al.* 2008; Pyšek *et al.* 2012), thereby disregarding the possibility that differences pre-dated invasion. Thus, a technique is needed to rapidly assess whether buckthorn is, or is not, altering soils.

Materials and methods

PAIRED-POINT RATIONALE AND OVERVIEW

We measured differences between paired buckthorn-invaded and buckthorn-free points in 10 soil parameters [response variables (RVs)] hypothesized to either respond to and/or promote invasions by buckthorn or other woodland plant invaders: relative water content (RWC), soil pH, soil Ca^{2+} , total C, total N, soil C : N ratios, soil nitrate-N ($\text{NO}_3^- - \text{N}$), soil ammonium-N ($\text{NH}_4^+ - \text{N}$), earthworm biomass and leaf litter mass (litter mass) (Wardle 2002; Gilbert & Lechowicz 2005; Frelich *et al.* 2006; Gilliam 2006; Heneghan *et al.* 2006; Heneghan, Steffen & Fagen 2007). We measured soil Ca^{2+} because buckthorn leaves have high levels of Ca^{2+} (Kraemer 1915) and because Ca^{2+} -rich leaf litter can increase soil- Ca^{2+} , soil pH and earthworm biomass, as well as decrease soil C : N ratios (Reich *et al.* 2005). We then modelled these differences (ΔRVs) in response to the level of invasion severity (defined below) found at each invaded point of all point pairs. A relationship (linear or nonlinear) between a ΔRV and severity, that is slope $\neq 0$, suggests a buckthorn-induced change, and an intercept (value of ΔRV where severity = 0) different from zero suggests a pre-existing difference (Fig. 1). Relating the magnitude of differences in RVs to that of invasion severity, that is an invasion gradient, had two benefits. First, unlike simply comparing differences between soils with and without exotic shrubs, it can provide evidence as to whether or not these differences pre-date invasion or are shrub-induced. Secondly, unlike short-term manipulative experiments, it can detect shrub-induced changes that accumulate slowly.

This framework assumes that the effects of an invader are proportional to, or occur at some threshold of (i.e. can be nonlinear), invader dominance – and not simply in response to invader presence. This assumption is justified, as buckthorn is hypothesized to alter soils via leaf litter inputs that likely increase in very small increments due to the slow rates at which the abundance of woody invaders increases within woodlands (Martin, Canham & Marks 2009; Iannone, Zellner & Wise 2014), and the known effects that adding high-quality leaf litter can have on soils (Reich *et al.* 2005). Small incremental increases in leaf litter inputs coupled to the inclusion of sample points having extremely low buckthorn abundance within our invasion gradient (see Table S1.1 of Appendix S1, Supporting information) improved

our ability to detect thresholds at which potential nonlinear effects might occur (e.g. Elgersma & Ehrenfeld 2011). Sampling along this gradient also allowed us to detect indirect below-ground effects such as those resulting from an invader causing either shifts in soil-microbial functionality or losses of native plants, as these changes can also be related to invader abundance (Ehrenfeld 2003; Wardle *et al.* 2011).

STUDY DESIGN

We established this study within the remnant woodlands of 15 natural areas (sites) spread throughout an approximately 5500-km² region extending 68 km north, 48 km south and 48 km west of downtown Chicago (Fig. S1.1 of Appendix S1, Supporting information). Each site lies within an urban/suburban matrix. Buckthorn invasions within each site are patchy, suggesting spatial heterogeneity in conditions suitable for buckthorn. Within each site, we located from three to 15 buckthorn-invaded sample points that varied in invasion severity (defined below), and paired each to an uninvaded reference point located between 28 to 140 m away, generating 97 invaded–uninvaded point pairs (194 total points). Buckthorn-free points had no known history of buckthorn invasion. We then measured each RV and the vegetation at all points between 11 May and 11 September 2009. Soils at most points were Alfisols (148 points); soils at the remaining points were either Mollisols (26 points), unmapped (14 points) or Inceptisols (three points). See Table S1.2 of Appendix S1 (Supporting information) for soil taxonomy, coordinates, elevation and slope of all sample points.

To ensure that our conclusions applied to multiple woodland and soil types, we selected the woodlands containing sample points without considering differences in plant communities or soils that might exist within these woodlands, or between the points of each point pair; however, we documented these differences. Tables S1.3 and S1.4 of Appendix S1 (Supporting information) provide a summary of the non-buckthorn woody and herbaceous vegetation found at each sample point.

SAMPLING BELOW-GROUND PARAMETERS

Soil parameters more reflective of long-term change, including pH, total C, total N, C : N ratio, soil Ca^{2+} and earthworm mass

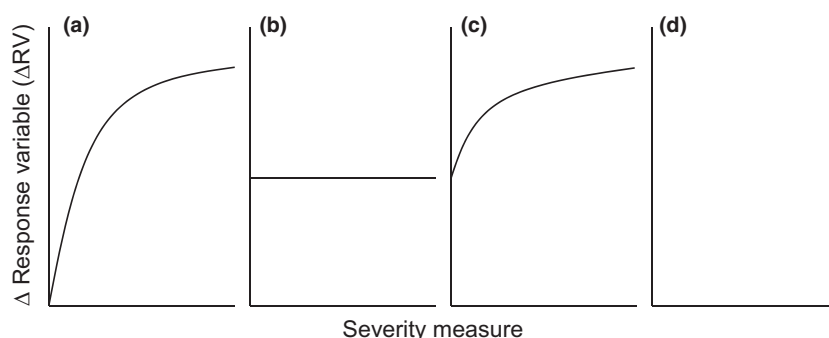


Fig. 1. Conceptualization of the paired-point framework. ΔRV = invaded – uninvaded values. Relationship a (intercept = 0 and slope > 0) suggests a buckthorn-induced change. Relationship b (intercept > 0 and slope = 0) suggests a pre-invaded difference that promotes establishment. Relationship c (intercept > 0 and slope > 0) suggests a pre-invaded difference that promotes establishment and a buckthorn-induced change after establishment. Relationship d (intercept = 0 and slope = 0) suggests neither pre-invaded difference nor buckthorn-induced change. These are four likely relationships; others may exist.

(Stevenson & Cole 1986), were measured once during the spring [11 May to 5 June; sample period 1 (SP1)]. Those most likely to exhibit larger intra-annual variation, including RWC, $\text{NO}_3^- - \text{N}$, $\text{NH}_4^+ - \text{N}$ and litter mass, were measured during SP1, mid-summer [13 to 22 July (SP2)], and late-summer [31 August to 11 September (SP3)]. A 10×6 cm soil core (depth \times width) located randomly within 1 m of each sample point was collected and stored at -20 °C until processed. We collected earthworms and leaf litter from a randomly placed 0.25×0.25 m (0.0625 m²) plot located within 2 m of the sample points for 75 of the 97 point pairs. Points belonging to the same point pair were sampled on the same day. See Appendix S2 (Supporting information) for justification of sample design and analytical procedures. See Table S1.5 of Appendix S1 (Supporting information) for summary statistics for RV values across all sample points and Δ RV values across all point pairs.

QUANTIFYING INVASION SEVERITY

We developed a severity measure to quantify the likely impact of buckthorn at each invaded point. The severity measure was based upon components of buckthorn dominance [basal area, cover, sapling density (stems with diameters < 1 cm) and height] and age measured in the 100 m² area surrounding each invaded point from mid-June through mid-August 2009 (summarized in Table S1.1 of Appendix S1; see Appendix S2, Supporting information, for detailed methods). These values were used to calculate Gower's distances (Gower 1971) among all invaded points, which were then used to conduct a principal coordinates analysis (PCoA) (Gower 1966). An invaded point's severity measure was then defined as its score along the two major axes of this ordination, hereafter referred to as Severity Index 1 (SI1) and Severity Index 2 (SI2), which combined explained 83% of the total variation in the data (Fig. 2; Table S3, Supporting information).

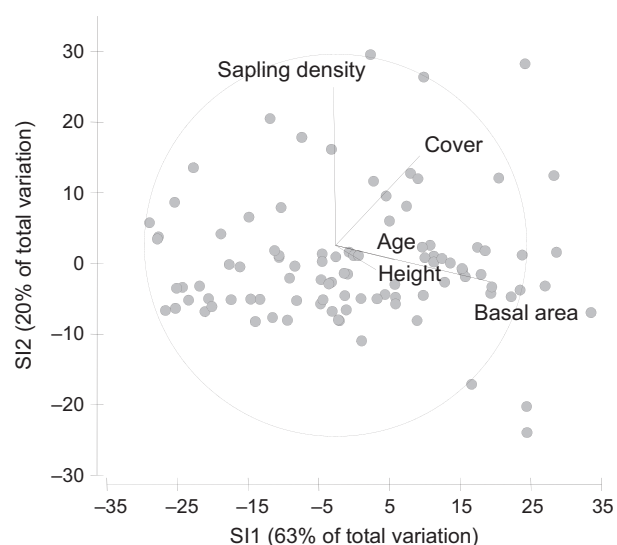


Fig. 2. The severity measure (PCoA) constructed from measures of buckthorn at each invaded point. Vectors show the results of the partial vector analysis (r). Circle depicts $r = 1.0$. Age is only weakly related to SI1 and SI2; thus, its vector is difficult to see. The severity measure of an invaded point is its score along both SI1 and SI2.

Creating the severity measure simplified our statistical analyses (described below), and more importantly, made ecological sense. Most buckthorn measures, because they covaried and were skewed, did not meet the assumptions of multiple regression (Zuur, Ieno & Elphick 2009) (Table S1.1 of Appendix S1, Supporting information). Merging these values into two ordination axes alleviated this complication and decreased the likelihood of Type I errors by reducing the number of terms in our statistical models from five main effects (i.e. each buckthorn measure), plus potential interactions, to just two. Modelling Δ RVs in response to invasion age alone was insufficient, as some older invasions exhibited low buckthorn dominance and *vice versa*.

A partial vector analysis (Anderson, Gorley & Clarke 2008) revealed that the indices of the severity measure (SI1 and SI2) each communicate an ecologically meaningful, and separate, aspect of buckthorn structure (Fig. 2; Table S3, Supporting information). Basal area, height and invasion age were all positively related to SI1, but negatively and weakly related to SI2. Sapling density was positively related to SI2. Cover was positively related to both SI1 and SI2. Thus, increases in SI1 and SI2 reflect long-term vs. short-term changes in buckthorn dominance, respectively (i.e. changes in basal area, height and age vs. changes in sapling density). The negative relationships of basal area, age and height to SI2 simply reflect the decrease in buckthorn saplings that often occurs with increasing buckthorn dominance (Table S1.1 of Appendix S1, Supporting information), that is density dependence (Hille Ris Lambers, Clark & Beckage 2002).

TESTING FOR PRE-EXISTING DIFFERENCES AND BUCKTHORN-INDUCED CHANGES

We modelled differences between the points of all point pairs in each RV (Δ RV) as a response to both SI1 and SI2 using linear regression (exceptions noted below). We constructed separate models for each sample period in which a Δ RV was measured. Non-significant model terms were removed from models when doing so did not increase AIC values by more than 2 (Burnham & Anderson 2002). Removing non-significant terms was necessary, as doing so affected estimates of model intercepts (i.e. pre-invaded differences). All invaded points had some buckthorn; thus, SI values never truly equalled zero. Determining at what distance beyond the lowest SI value true zero occurred would have been subjective. Therefore, we estimated model intercepts as the predicted Δ RV value where the SIs included in the model were at their lowest values.

Preliminary analyses revealed that including site as a random effect in statistical models did not affect the interpretation of our analyses, suggesting the absence of spatial autocorrelation at the regional scale. The heterogeneity in both soils and plant communities within individual sites further justified excluding site from our statistical models. Preliminary semi-variograms also revealed no evidence that the scores of invaded points along either SI1 or SI2 were spatially autocorrelated within sites.

We were unable to analyse some Δ RVs using linear regression. $\Delta\text{NH}_4^+ - \text{N}$ was modelled as a cubic regression spline with its gamma parameter (γ) set to 1.4 to prevent exaggerations of nonlinearity (Wood 2006). Both $\Delta\text{NO}_3^- - \text{N}$ and Δ earthworm mass violated assumptions of parametric statistics. Therefore, we used ranked regressions to detect significant relationships ($P < 0.10$) (Conover & Iman 1981) and bootstrapping to

estimate values for statistically significant model terms by randomly re-sampling paired values for the model term and the ΔRV with replacement 1000 times (Keele 2008). We considered bootstrapped estimates statistically significant when their 95% bias-corrected, accelerated CI did not encompass zero (Efron & Tibshirani 1986).

To limit the likelihood of Type I errors often caused by conducting many separate univariate analyses (Legendre & Legendre 2012) and to account for the likely covariance among ΔRV s (Wardle 2002), we first tested for system-level differences between buckthorn-invaded and buckthorn-free points using both permutational analysis of variance and a test for homogeneity of dispersion, and then tested whether these system-level differences were related to invasion severity (i.e. were buckthorn-induced) using a Mantel test. These tests revealed evidence of both pre-existing and buckthorn-induced differences (see Appendix S4 for details, Supporting information), justifying the separate analysis of each ΔRV described above.

Finally, to determine the contribution of each RV to the below-ground variation among point pairs, we used the ΔRV values measured for each point pair to calculate Gower's distances among point pairs, and then used these distances to conduct a PCoA and partial vector analyses (Gower 1966, 1971; Anderson, Gorley & Clarke 2008).

PCoAs and partial vector analyses were conducted using PERMANOVA + v. 1.0.2 (Anderson, Gorley & Clarke 2008). ΔRV s were modelled in response to SII and SI2 using R v. 2.14.1 (R Core Team 2014).

Results

The estimated parameters ($\pm SE$) of the most parsimonious model for each ΔRV are shown in Table 1. Even with the large number of point pairs ($N = 75$ or 97), no model explained more than 16% of the variation in a ΔRV . Nonetheless, modelling ΔRV s in response to an invasion gradient revealed insights not possible from simply comparing the magnitude of the differences between buckthorn-invaded and buckthorn-free points.

Most ΔRV s were best modelled solely as an intercept that was significantly greater than zero, that is, SII and SI2 explained no variation and were removed from the model (Table 1), suggesting that the majority of differences between invaded and uninvaded soils pre-dated invasion and were not buckthorn-induced. Soil pH was 0.30 ± 0.07 higher, soil Ca^{2+} was $1100 \pm 180 \mu g g^{-1}$ higher, total N was $0.12 \pm 0.02\%$ higher, total C was $1.60 \pm 0.28\%$ higher, and RWC was from $11.0 \pm 2.0\%$ to $7.0 \pm 1.4\%$ higher in invaded than uninvaded soils (mean $\pm SE$).

Throughout the growing season, $NH_4^+ - N$ was from 2.17 ± 0.33 to $4.82 \pm 1.95 \mu g g^{-1}$ higher in invaded than uninvaded soils. Modelling the data revealed that differences likely pre-dated invasion and were not buckthorn-induced. $\Delta NH_4^+ - N$ at SP1 was best modelled as a

Table 1. Parameter estimates ($\pm SE$) of the most parsimonious model for each ΔRV

Δ Response Variable (ΔRV)	Model terms				
	Intercept	SII	SI2	Dist [†]	Var. Explained [‡]
ΔpH	$0.30 \pm 0.07^{****}$				
ΔRWC SP1	$0.11 \pm 0.02^{****}$				
ΔRWC SP2	$0.08 \pm 0.02^{****}$				
ΔRWC SP3	$0.07 \pm 0.01^{****}$				
$\Delta Litter$ SP1 ($g\ 0.0625\ m^{-2}$)	$7.80 \pm 3.50^*$	$-0.39 \pm 0.10^{***}$			0.16
$\Delta Litter$ SP2 [§] ($g\ 0.0625\ m^{-2}$)	$-1.60 \pm 1.60\ ns$	$-0.13 \pm 0.06^*$			0.05
$\Delta Litter$ SP3 ($g\ 0.0625\ m^{-2}$)	$0.74 \pm 2.30\ ns$	$-0.17 \pm 0.06^*$			0.07
$\Delta Earthworm$ [¶] ($g\ 0.0625\ m^{-2}$)	$-0.20 \pm 0.19\ ns$			$0.44 \pm 0.23^*$	0.05
$\Delta Total\ C$ (%) [§]	$1.60 \pm 0.28^{****}$				
$\Delta Total\ N$ (%)	$0.12 \pm 0.02^{****}$				
$\Delta C : N$ ratio [§]	$-0.15 \pm 0.23\ ns$				
ΔCa^{2+} ($\mu g\ g^{-1}$)	$1100 \pm 180^{****}$				
$\Delta NH_4^+ - N$ SP1 ^{††} ($\mu g\ g^{-1}$)	$4.80 \pm 2.00^*$	nl, ns (See Fig. 3a)			0.05
$\Delta NH_4^+ - N$ SP2 ($\mu g\ g^{-1}$)	$2.50 \pm 0.40^{****}$				
$\Delta NH_4^+ - N$ SP3 ($\mu g\ g^{-1}$)	$2.17 \pm 0.33^{****}$				
$\Delta NO_3^- - NSP1$ [¶] ($\mu g\ g^{-1}$)	$-0.41 \pm 0.40\ ns$	$-0.015 \pm 0.007^*$	$0.030 \pm 0.013^*$		0.07
$\Delta NO_3^- - NSP2$ [¶] ($\mu g\ g^{-1}$)	$-0.11 \pm 0.27\ ns$				
$\Delta NO_3^- - NSP3$ [¶] ($\mu g\ g^{-1}$)	$-0.19 \pm 0.15\ ns$				

Dist = physical distance between points of a point pair; nl = nonlinear relationship.

ns = $P > 0.10$; * = $P \leq 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$.

[†]Dist added to initial models for $\Delta total\ C$, $\Delta total\ N$, $\Delta soil\ Ca^{2+}$, $\Delta earthworm\ mass$ and $\Delta NH_4^+ - N$ at SP3, as Spearman correlations revealed this parameter to be potentially related to these ΔRV s ($P \leq 0.16$).

[‡]Variance explained by model terms.

[§]Model allowed for heterogeneous variance across SII (Zuur *et al.* 2009).

[¶]The significance of model terms and the variance they explained were determined via ranked regression. Bootstrapping was then used to validate significance and to estimate model parameters.

^{††}Intercept was assumed to differ significantly from zero when the 95% CI of predicted value where SII was at its lowest value did not encompass zero.

nonlinear, but non-significant, response to SI1 ($P = 0.31$) with an intercept significantly greater than zero (Table 1; Fig. 3a). $\Delta\text{NH}_4^+ - \text{N}$ values at SP2 and SP3 were best modelled as intercepts only, both of which were significantly greater than zero (Table 1).

Δ Litter mass during all sample periods was negatively related to SI1 (Table 1; Fig. 3b–d), suggesting that buckthorn accelerates leaf litter decomposition. The intercept of the model for Δ litter mass at SP1, but for no other sample period, was significantly greater than zero (Table 1; Fig. 3b–d), suggesting that buckthorn is more likely to establish in areas having more leaf litter

in the spring as well as higher rates of leaf litter decomposition.

$\Delta\text{NO}_3^- - \text{N}$ was negatively related to SI1 and positively related to SI2 during SP1, but was not related to either severity index during other sample periods (Table 1; Fig. 3e,f), suggesting that buckthorn affects $\text{NO}_3^- - \text{N}$, but only in the spring. Bootstrapping confirmed the significance of these relationships (95% CI for slope of SI1 = -0.028 , -0.002 ; 95% CI for slope of SI2 = 0.007 , 0.058), and further revealed that the intercepts for all $\Delta\text{NO}_3^- - \text{N}$ models did not differ significantly from zero (95% CI for SP1 = -1.18 , 0.45 ; 95%

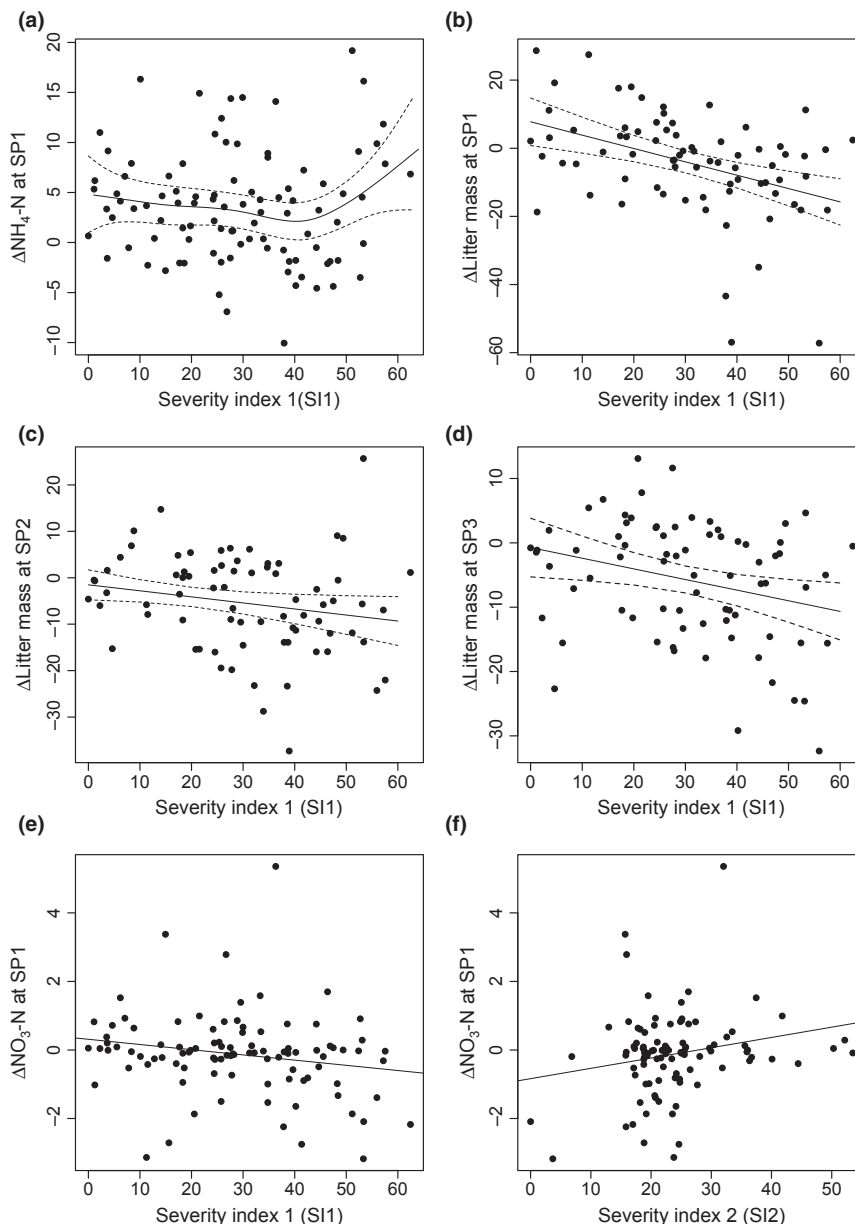


Fig. 3. The effect of buckthorn on below-ground properties: (a) the nonlinear, non-significant relationship between SI1 and $\Delta\text{NH}_4^+ - \text{N}$ in spring (SP1) ($P = 0.31$); the negative relationship between SI1 and Δ Litter mass at (b) SP1 ($P = 0.0003$), (c) mid-summer (SP2; $P = 0.04$) and (d) late-summer (SP3; $P = 0.02$); and the (e) negative and (f) positive relationships between $\Delta\text{NO}_3^- - \text{N}$ at SP1 and SI1 and SI2 ($P = 0.02$ and $P = 0.06$, respectively).

CI for SP2 = $-0.70, 0.37$; 95% CI for SP3 = $-0.54, 0.05$) (Table 1), suggesting that $\text{NO}_3^- - \text{N}$ did not differ between invaded and uninvaded soils prior to invasion.

We detected no effects of buckthorn on either soil C : N ratios or earthworm mass, as well as no differences between these RVs in invaded and uninvaded soils prior to invasion (i.e. SI1 and SI2 explained no variation and model intercepts did not differ significantly from zero; Table 1).

The PCoA revealed that 68% of the variation among point pairs was explained by two axes (PCoA1 = 57%; PCoA2 = 12%) (Fig. 4). ΔCa^{2+} was strongly related to PCoA1 ($r = -0.91$), whereas Δ litter mass during all sample periods and $\Delta\text{NH}_4^+ - \text{N}$ during SP1 were only weakly related to PCoA2 ($|r| \leq 0.42$) (Fig. 4). Analyses in which the potentially influential point pair located near ' ΔCa^{2+} ' in Fig. 4 was removed revealed the same pattern (Table S5, Supporting information).

Discussion

APPLIED UTILITY OF 'PAIRED-POINT' FRAMEWORK

Efforts to control invasive species are challenging, costly and can even have unanticipated consequences (Zavaleta, Hobbs & Mooney 2001), necessitating a clear understanding of whether or not such efforts are needed and/or likely to be beneficial (Hulme 2006). Our 'paired-point' framework can help with these decisions. By being able to detect below-ground consequences across a wide range of environmental conditions, our framework can help managers evaluate overall invader impacts (*sensu* Parker *et al.* 1999), thereby helping them prioritize control efforts and to assess the need for pre-restoration soil remediation. By being able to detect below-ground differences that pre-date and possibly promote invasion, this framework can further help identify which below-ground factors are related to which invasive plant species, enabling managers to develop more-targeted monitoring programmes.

Finally, these insights can be generated relatively quickly, allowing managers to rapidly incorporate them into their management programmes.

With regard to our investigation, we found evidence that the elevated levels found in buckthorn-invaded soils for most of the measured factors (soil moisture, pH, total carbon, total nitrogen, calcium and soil $\text{NH}_4^+ - \text{N}$) are not buckthorn-induced, but rather pre-date and possibly promote invasion. We also discovered that buckthorn invasions are not associated with either decreased soil C : N ratios or increased earthworm biomass. These patterns contradict current hypotheses about the consequences of this invader (Heneghan *et al.* 2006; Heneghan, Steffen & Fagen 2007), suggesting that managers should not base decisions regarding regional buckthorn control on concerns about what this species is or is not doing to these soil parameters and that post-buckthorn-removal soil remediation (e.g. soil mulch amendments) will not benefit restorations, the latter being confirmed by a recent field experiment (Iannone *et al.* 2013). Nonetheless, knowing that buckthorn preferentially establishes in soils having these characteristics can help to develop better focused monitoring programmes, aiding in the prevention of further buckthorn spread.

Our paired-point framework did, however, reveal evidence of at least some buckthorn-induced changes. Our results suggest that buckthorn establishes in areas with greater spring leaf litter mass and greater rates of leaf litter decomposition, and then accelerates decomposition further and alters spring levels of soil $\text{NO}_3^- - \text{N}$. Concerns from managers regarding buckthorn's impacts on these soil parameters should therefore be accounted for when devising regional management plans. Managers should also focus monitoring efforts on areas likely to have higher spring leaf litter and decomposition rates.

These insights would not have been possible from simple comparative or short-term manipulative studies, illustrating this framework's utility for investigating the below-ground causes and consequences of slow-growing

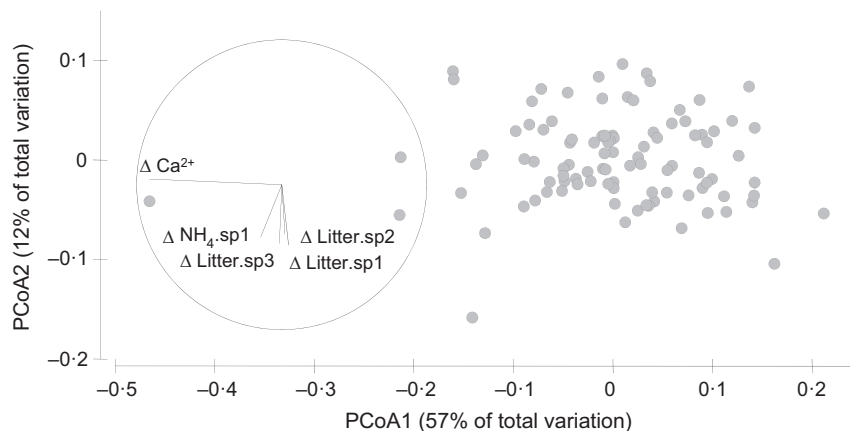


Fig. 4. PCoA and partial vector analysis determining the relatedness of each Δ RV to the variation among point pairs. Only vectors having $|r| \geq 0.30$ are shown. Circle depicts $r = 1.0$. Analyses conducted both with and without the potentially influential point pair located below ' ΔCa^{2+} ' revealed the same pattern (Table S5, Supporting information).

invasive plants. This framework also possesses benefits over other empirical approaches. Unlike common-garden and possibly mesocosm studies, it can generate insights from a single season of data. Using below-ground changes that occur after invasive plant removal to infer below-ground effects may be flawed as these changes may not occur in the direction of pre-invaded conditions (Suding, Gross & Houseman 2004). Sampling across *in situ* invasion fronts may also be of limited use, as one may not know whether the variability occurring across these fronts pre-dates invasion. Even when pre-invaded conditions are known, one still needs to wait for expansion in order to detect changes. Many of these methods also have a more limited inference space (*sensu* Manly 1992) due to controlled conditions, low replication and/or limited spatial scope.

POTENTIAL IMPROVEMENTS

Despite these benefits, improvements are possible. For example, as buckthorn was present at all invaded points, statistical models did not extrapolate beyond the lowest levels of severity found at these points. Therefore, evaluating model intercepts requires some caution when a model has both an intercept not significantly different than zero and a statistically significant severity index. This complication may be avoided by sampling from a set of uninvaded point pairs to better estimate below-ground differences when invasion severity truly equals zero. Additionally, for both ecological and statistical necessity, we merged measures of buckthorn dominance and age (i.e. created SI1 and SI2). Avoiding such a merger whenever possible would have practical advantages. For example, it would be much easier for managers to visually assess the potential below-ground effects of an invader if these effects were related to parameters such as basal area or density rather than scores along ordination axes.

GUIDING FUTURE RESEARCH

Our paired-point framework can guide the future manipulative (and likely long-term) experiments needed to confirm the below-ground causes and consequences of slow-growing invasive plants. For example, since using this framework revealed evidence that total C, total N, RWC, pH, Ca^{2+} and $\text{NH}_4^+ - \text{N}$ were likely greater in invaded soils prior to invasion, studying these factors in the context of buckthorn establishment rather than in the context of buckthorn-induced change would likely be more fruitful. Our findings also suggest several new research directions.

Our results point to soil Ca^{2+} as an important and yet unrecognized driver of woodland shrub invasion. Soil Ca^{2+} was not only higher in buckthorn-invaded soils, but was also much more strongly related to the variation among point pairs than any other RV. Given this evidence, as well as similar patterns regarding woodland

plant invasions found by others (Harrison 1999; Rose & Hermanutz 2004; Gurevitch *et al.* 2008), it is surprising that the role of soil Ca^{2+} in driving woodland shrub invasions, or woodland plant invasions in general, and the mechanisms by which it does so have yet to be thoroughly investigated.

We found evidence that buckthorn reduces the leaf litter layer, as has been found for other invasive shrubs (Kourtev, Huang & Ehrenfeld 1999; Ashton *et al.* 2005). This loss can negatively affect understorey plants and both leaf litter and soil-dwelling organisms (Heneghan, Clay & Brundage 2002; Moore *et al.* 2004; Frelich *et al.* 2006). As the majority of woodland biodiversity occurs within these subsystems (Wardle 2002; Moore *et al.* 2004; Gilliam 2007), research is needed to determine whether shrub-induced leaf litter loss is a concern for conservation.

Invasive plants cannot only affect soils directly, but also indirectly by causing the loss of native species (Wardle *et al.* 2011). Although our framework cannot differentiate between these pathways, using it revealed evidence of the latter. Despite buckthorn's high-N leaf litter, spring $\text{NO}_3^- - \text{N}$ decreased in relation to long-term changes in buckthorn dominance (i.e. in relation to SI1). This unexpected finding may be explained by the decreased plant abundance observed beneath buckthorn canopies (Mascaro & Schnitzer 2007), leading to decreased system-level uptake of $\text{NO}_3^- - \text{N}$ and subsequent denitrification and leaching (Stevenson & Cole 1986; Muller 2003). From this finding, it is clear that a greater understanding of the indirect below-ground effects of woodland shrub invasions is needed.

Perhaps the most compelling question raised by our study is why we detected so few buckthorn-induced changes despite buckthorn's high-N and high- Ca^{2+} leaf litter (Kraemer 1915; Heneghan, Clay & Brundage 2002), and the ability of plants possessing these traits to alter soils (Ehrenfeld, Kourtev & Huang 2001; Kourtev, Ehrenfeld & Häggblom 2003; Reich *et al.* 2005). Elgersma *et al.* (2011) found that planting another invasive shrub having N-rich leaf litter (Japanese barberry *Berberis thunbergii*) in soils of an uninvaded woodland also had no detectable below-ground effects after 2 years, despite observing effects within the same period in greenhouse studies (Ehrenfeld, Kourtev & Huang 2001; Kourtev, Ehrenfeld & Häggblom 2003). Elgersma *et al.* (2011)'s study shows that soils may exhibit short-term resistance to shrub-induced changes, while our study shows that this resistance may be long-lived. Investigations into why at least some soils are resistant to shrub-induced changes are therefore needed.

BROADER IMPLICATIONS FOR DETECTING BELOW-GROUND EFFECTS

Our investigation also yielded valuable insights into the detection of below-ground consequences of invasive plants

in general. First, we found that both the degree to which invaded and uninvaded soils differ and the relationship of this difference to invasion severity can vary intra-annually. Therefore, intra-annual sampling may be needed to detect changes in below-ground factors known to fluctuate within a growing season. We should also note that the growing season in which we sampled was unusually cool and wet (Table S1.6 of Appendix S1, Supporting information). While this variation in weather likely had little effect on most of the measured RVs (Stevenson & Cole 1986; Edwards 2004), future studies may benefit from sampling across multiple years.

Even with 97 point pairs, statistical models explained a small fraction of the variability in Δ RVs. This high level of variability reaffirms the inability to make broad generalizations about the effects of invasive plants from studies having low replication and/or small spatial reference (Hulme *et al.* 2013). For example, despite other studies finding associations between exotic earthworms and invasive shrubs (Kourtev, Huang & Ehrenfeld 1999; Heneghan, Steffen & Fagen 2007; Madritch & Lindroth 2009), and the hypothesis that exotic earthworms facilitate plant invasions (Kourtev, Huang & Ehrenfeld 1999; Frelich *et al.* 2006), our investigation revealed no such pattern, likely due to its regional scale and much higher replication relative to prior investigations. This high level of variability also suggests the need for more-localized investigations, under more precise environmental conditions, as a way to confirm the robustness of our findings (i.e. to determine whether buckthorn does affect soil parameters, but only under restricted conditions).

Our investigation confirmed the utility of relating the magnitude of differences between areas with and without invasive plants to gradients in invasion dominance (Hulme *et al.* 2013). Doing so revealed evidence that buckthorn is not as much of a 'driver' of below-ground change (*sensu* MacDougall & Turkington 2005) as hypothesized from studies employing simple below-ground comparisons (Heneghan *et al.* 2006; Heneghan, Steffen & Fagen 2007). Three implications emerge as follows: (i) simple comparative studies should not be included in meta-analyses aimed at detecting the overall effects of invasive plants (e.g. Liao *et al.* 2008; Pyšek *et al.* 2012); (ii) any differences between soils with and without invasive plants detected via simple comparisons should be considered both equally as potential causes and consequences of the invasion, until studies under more controlled conditions suggest otherwise; and (iii) it can be misleading to cite the results of studies using simple comparisons as definitive proof of invader-induced effects.

Collectively, our findings suggest that a greater consideration of how the below-ground differences between areas with and without invasive plants relate to establishment is needed. The lack of research on this question is surprising, given the long-recognized relationship between variability in soil properties and woodland plant community composition at both local and regional scales

(e.g. Salisbury 1920; Curtis 1959; Grimm 1984 to name but a few). Because invasions provide a valuable opportunity to investigate both the factors that regulate species distributions and the characteristics of communities that promote change (Williamson 1996), such consideration will inevitably improve our understanding of both woodland invasions and the factors that promote change in woodland communities, enabling us to better manage and limit the spread of these invasions.

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Data accessibility

Estimates of buckthorn abundance and invasion age for the invaded points of all point pairs, as well as the scores of these points along S11 and S12; RV values for all sample points; and Δ RV values for all point pairs can be found at the Dryad Digital Repository: DOI: doi:10.5061/dryad.vb120 (Iannone *et al.* 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Descriptions of vegetation, location, elevation, slope, and soils for study sites and sample points.

Table S1.1. Summary of, and co-variance among, the individual measures of buckthorn invasion used to construct the Severity Measure.

Table S1.2. Coordinates, elevation above sea level, slope, soil type, and soil taxonomic classification for each point in all point pairs.

Table S1.3. Abundance and frequency of non-buckthorn woody species found across all invaded and uninvaded points.

Table S1.4. Summary of understorey plant cover for the plant functional groups (non-buckthorn) found across all invaded and uninvaded points.

Table S1.5. Summary statistics for (A) Δ RV values (invaded – uninvaded) across all point pairs and for (B) response variables (RVs) across all sample points (invaded and uninvaded).

Table S1.6. Comparison of the regional climatic conditions for the 2009 growing-season (March–November) to the long-term regional climatic conditions from 1945–2008.

Fig. S1.1. The location of the 15 natural areas (sites) used for the paired-point study.

Appendix S2. Methods for quantifying soil parameters, vegetation structure of buckthorn, invasion age, and structure and composition of the non-buckthorn vegetation.

Table S3. Numerical output for (A) PCoA and (B) follow-up partial vector overlays used to construct and interpret the Severity Measure (see Fig. 2 of main paper).

Appendix S4. Methods and results for preliminary multivariate statistical analyses used to test for pre-existing and buckthorn-induced differences at the system level.

Table S4.1. Results of preliminary multivariate tests for pre-existing differences and buckthorn-induced changes at the system level.

Table S5. Numerical descriptions of PCoA and partial vector analysis used to determine the relatedness of each Δ RV to the variation among point pairs.